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MICROBIAL COMPOSITION OF INFECTIOUS PROCESSES OF ENDODONTIC ORIGIN: AN INTEGRATIVE REVIEW

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ABSTRACT

The objective of the present study was to analyze the variety of microbial composition present in infectious processes of endodontic origin, through an integrative review of the literature. He found a dominance of Gram-negative and anaerobic bacteria in the microbial composition of infectious processes of endodontic origin. The study consisted of an integrative literature review, a method that provides the synthesis of knowledge and the combination of data from the theoretical and empirical literature, in addition to incorporating a wide range of purposes, such as defining concepts, reviewing theories and evidence, and analyzing methodological problems of a specific topic. For the development of the integrative review, it began with the definition of the guided question, constructed through the PICO strategy, acronym for Population (P), Intervention (I), Comparator (C) and Outcome (O), as shown in Chart 1. Thus, our guiding question was "What is the microbial composition and variation found in endodontic infectious processes. It was concluded that bacteria such as E. faecalis, Gram-positive, facultative anaerobic, can represent great risks,

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especially due to antibiotic resistance. In addition, evidence was observed in the literature regarding the composition of viruses and fungi in these infections; However, evidence is still scarce and, therefore, more studies of greater methodological rigor and sample size are needed.

Keywords: Endodontics. Endodontic Infection. Microbiology.

INTRODUCTION

The oral microbiota is characterized by millions of microorganisms that relate harmoniously in order to promote homeostasis1-3. However, a metabolic imbalance influenced by external actions can trigger pathological processes that, if not treated, can culminate in necrosis of the pulp tissue and development of odontogenic lesions.

The microorganisms present in the root canal system must always be in balance, as they directly influence the inflammatory reaction of the periapical tissues as well as their symptoms. If the microorganisms reach the root canal systems, they will be defined as potential endodontic pathogens and, in turn, if they reach the apical connective tissues, they will cause an inflammatory response at the site4. In these cases, the intervention of choice is the endodontic approach with the objective of treating this imbalance in the canal, promoting its disinfection and, in turn, the maintenance of the tooth in the oral cavity5.

However, the potentiation of microbial activity from an untreated external stimulus may generate pathogenic scenarios that will later determine clinical conditions of endodontic infection, culminating mainly in the formation of abscesses. Abscesses are characterized by microbial migration that is in an inflamed root canal to the periapical and periodontal tissues, disseminating the infectious process in these places5.

Persistent root apical lesions are mainly caused by strictly facultative anaerobic bacteria, such as Enterococcus faecalis. These bacteria can cause chronic apical periodontitis, which can lead to persistent root and extraradicular infections [5]. The anaerobic bacteria present in endodontic infections can be classified into two main groups: gram-positive anaerobic cocci, such as Enterococcus faecalis, Peptostreptococcus anaerobius, and Peptostreptococcus micros, and gram-negative anaerobic rods, such as Porphyromonas endodontalis, Porphyromonas gingivalis, Prevotella intermedia/nigrescens, and Prevotella melaninogenica6.

Different studies have been carried out seeking to evaluate the microbial composition associated with endodontic infectious processes, such as periapical abscesses, teeth with apical periodontitis, pulp necrosis and others. Therefore, understanding these processes, elucidating the wide range of microorganisms associated with endodontic infections, is essential to provide support for clinical decision-making, providing more favorable prognoses and greater predictability to cases.

In view of the above, the objective of the present study was to analyze the variety of microbial composition present in infectious processes of endodontic origin, through an integrative review of the literature.

MATERIALS AND METHODS

The present study consists of an integrative literature review, a method that provides the synthesis of knowledge and the combination of data from the theoretical and empirical literature, in addition to incorporating a wide range of purposes, such as defining concepts, reviewing theories and evidence, and analyzing methodological problems of a specific topic. For the development of the integrative review, we started by defining our guided question, constructed through the PICO strategy, acronym for Population (P), Intervention (I), Comparator (C) and Outcome (O), as shown in Chart 1. Thus, our guiding question was "What is the microbial composition and variation found in endodontic infectious processes?".

Table 1: Acronym CEECs

Acronym	Definition	Description
P	Population/Problem	Patients with endodontic infectious process
And	Exposure	Microorganisms of the root canal system
C	Comparison	Not applicable
Or	Outcome	Polymicrobial infections

Source: Prepared by the authors. Based on the methodological guidelines for the elaboration of a systematic review and meta-analysis of comparative observational studies on risk and prognostic factors of the Ministry of Health7

An electronic search was performed in the PubMed, VHL, and EMBASE databases between July and August 2023. The MeSH (Medical Subject Headings) was used to define the descriptors, in English, Portuguese and Spanish. The descriptors used were: "oral microbiome", "endodontic infection", "periradicular lesion", "periodontal abscess". Only studies published in English, Portuguese and/or Spanish, whose object of study was the application and outcome of intraoral reconstructive techniques in implant dentistry were included. Only studies published in the last 5 years were included. Case reports, animal studies, communications, theses, dissertations, unpublished manuscripts and publications that did not address the proposed problem were excluded. Of the articles analyzed, 20 were eligible and, therefore, included in this review.

The search strategy in each selected database is contained in Chart 2. After the electronic search, the references of all the articles found were organized in Zotero, which made it possible to exclude duplicate articles. The initial search retrieved a total of 261 studies after the removal of the duplicates. Data extraction was performed by two authors independently (L.G.S.D.J.) and (Y.G.L.S.), using the following predefined data fields: authors, design, year of publication, objective, sample, methods, evaluated factors, and results. Studies with missing important information were excluded. One of the authors was alleged to verify the extracted data for inconsistencies and attest that the eligibility criteria were met. Disagreements were resolved among the authors by discussion. The selection of studies was initially carried out through the reading of titles and abstracts, resulting in 44 studies, considering the previously established criteria. Finally, the full articles were read, and thus, 20 studies were included in the final sample of the research. In cases of disagreement, the publications were reviewed by all, and decisions were made by consensus.

The quality of the studies was evaluated according to the classification of the levels of evidence, namely: I: systematic review or meta-analysis of clinical trials; II: randomized controlled clinical trials; III: Controlled clinical trials without randomization; IV: Case-control and cohort; V: Systematic review of descriptive and qualitative studies; VI: Descriptive or qualitative studies (19). At the end of the categorization and analysis of the studies, the findings were interpreted.

Chart 2: Search Strategies

Database	Search strategy
PubMed	(("dental abscess"[All Fields] OR "odontogenic abscess"[All Fields] OR "periodontal abscess"[All Fields] OR "endodontic infection"[All Fields] OR "periradicular lesion"[All Fields]) AND "2013/05/09 00:00":"3000/01/01 05:00"[Date - Publication] AND (("microbiology"[All Fields] OR "oral microbiome"[All Fields] OR "polymicrobial infection"[All Fields]) AND "2013/05/09 00:00":"3000/01/01 05:00"[Date - Publication])) AND (y_5[Filter])
VHL	((((("dental abscess") OR ("odontogenic abscess")) OR ("periodontal abscess")) OR ("endodontic infection")) OR ("periradicular lesion")) AND ((("microbiology") OR ("oral microbiome")) OR ("polymicrobial infection")) AND (year_cluster:[2013 TO 2023])
EMBASE	('microbiology'/exp OR 'microbiology' OR 'oral microbiome'/exp OR 'oral microbiome' OR 'polymicrobial infection'/exp OR 'polymicrobial infection') AND ('dental abscess' OR 'odontogenic abscess' OR 'periodontal abscess' OR 'endodontic infection' OR 'periradicular lesion') AND [2018-2023]/py
Web of Science	Resultados para ((((("dental abscess") OR ("odontogenic abscess")) OR ("periodontal abscess")) OR ("endodontic infection")) OR ("periradicular lesion")) AND ((("microbiology") OR ("oral microbiome")) OR ("polymicrobial infection")) (Todos os campos) and 2023 or 2022 or 2021 or 2020 or 2019 or 2018 (Anos da publicação)

Source: Prepared by the authors.

RESULTS

The initial research resulted in 261 studies, of which 47 were obtained after the duplicates were removed. A total of 44 studies were evaluated for eligibility after reading the titles and abstracts, of which 24 were excluded because they did not fit the inclusion criteria. Therefore, considering both searches, a total of 20 articles were found eligible for inclusion in this review (Figure 1). Six (6) studies from Asia were included and conducted in Japan, India, Iran, and China. In addition, nine (9) European studies from five countries were included: the Netherlands, Switzerland, Germany, the United Kingdom, and Greece. Five (5) American studies were included in the sample, conducted in Canada, Brazil, the United States of America, and Mexico. Regarding the study design, 16 were randomized controlled trials/prospective clinical studies (80%), 3 were cross-sectional studies (15%), and 1 cohort study (5%).

Figure 2. Flowchart of the title/abstract and full text screening process for selection of articles included in the review.

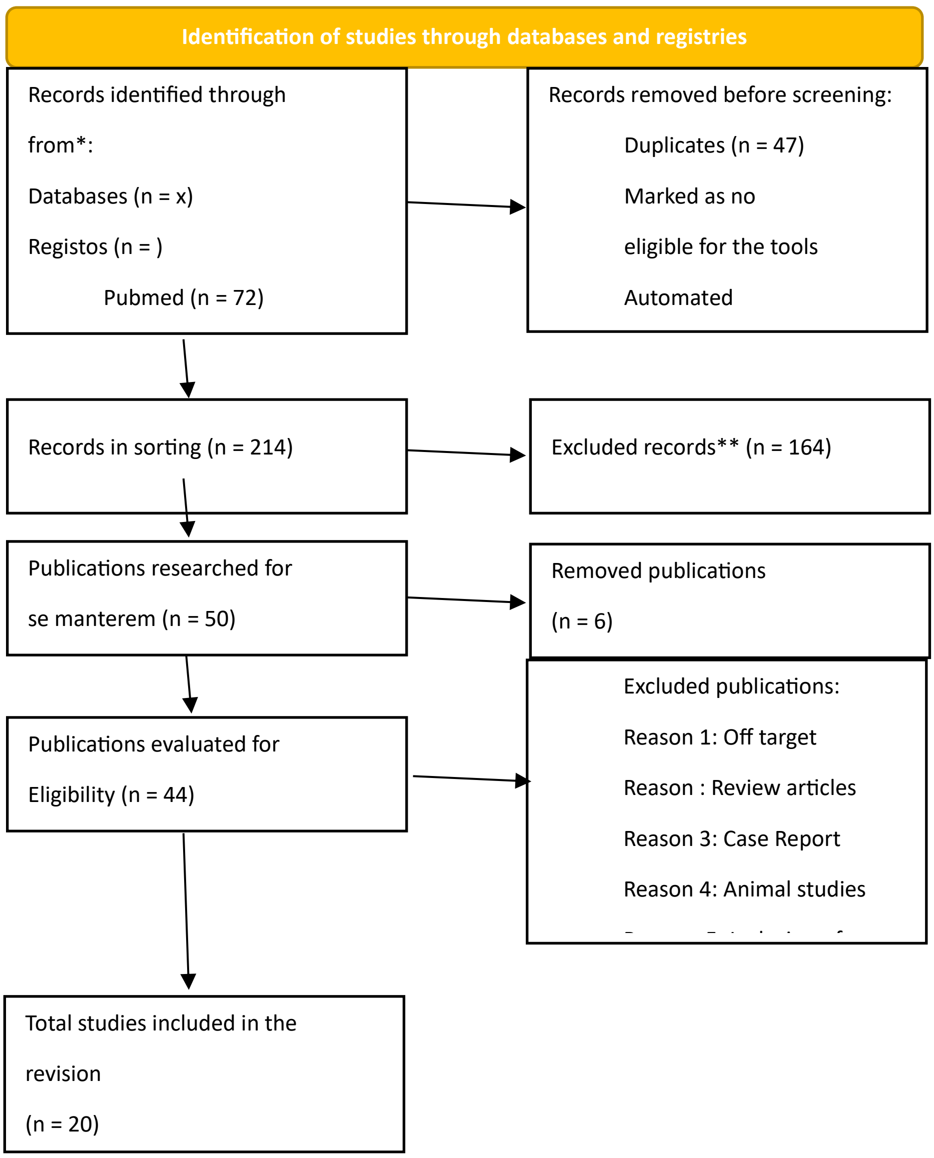

Table 3: Classification of studies according to authors/year, place of study, study design, sample, factors evaluated, and main results.

General characteristics of the studies included in the review that evaluated the microbiota of endodontic infectious processes						
Author	Country	Design	Level of evidence	Sample size	Sampling site	Main results
Jimenez et al., 2022 8	Chile	Cross-sectional study	SAW	80 patients	Periodontite apical	The intracanal detection of P. endodontalis and P. gingivalis in PsA was 33.3% and 22.9%, respectively.
Amaral et al., 2022 9	USA	Clinical study	III	25 patients	Asymptomatic apical periodontitis	High bacterial diversity in the microbiome of the teeth analyzed. Firmicutes (27%), Bacteroidetes (21%), Proteobacteria (21%) and Actinobacteria (12%) were more prevalent. The bacterium Bacteroidaceae [G-1] HMT 272 was the most prevalent and abundant phylotype.
Gaeta et al., 2023 10	Switzerland	Cohort	IV	67 patients	Healthy vital teeth, healthy teeth treated without injury, irreversible pulpitis, necrosis, and post-treatment apical periodontitis	E. faecalis was found in 18 root canal and saliva samples. Root canal isolates of E. faecalis were recovered with higher frequency of post-treatment apical periodontitis.
Kesim et al., 2023 11	Germany	Clinical study	III	20 teeth	Teeth with primary endodontic infection and persistent endodontic infection	The facultative anaerobic Gram-negative class Gammaproteobacteria, two orders (Pasteurellales, Vibrionales) and two families (Pasteurellaceae, Vibrionaceae) were more abundant in the primary endodontic infection group. The Gram-positive bacteria of the order Actinomycetales and Gram-positive anaerobic taxa, one genus (Olsenella) and one species (Olsenella uli) were more abundant in the persistent endodontic infection group.
Brzezińska -	Canada	Randomized controlled trial	II	64 samples	Root canal of patients	Archaea was detected in 48.4% of the samples. The main representative of the Archaea

Błaszczyk et al., 2018 12					with and after treatment of primary endodontic infection	domain found in the infected pulp tissue was Methanobrevibacter oralis.
Pourhajibagher et al., 2018 13	Holland	Clinical study	III	36 patients	Primary endodontic infections	45,4% dos isolados bacterianos eram anaeróbios estritos, incluindo Veillonella parvula, Porphyromonas gingivalis, Propionibacterium acnes, Lactobacillus acidophilus, Campylobacter rectus e Slackia exigua; 45,4% eram anaeróbios facultativos; e 9,2% eram bactérias microaerófilas (Aggregatibacter actinomycetemcomitans).
Pourhajibagher et al., 2018 14	Holland	Clinical study	III	50 patients	Primary and secondary endodontic infections	Enterococcus faecalis (100%) foi o mais frequente, seguido por Veillonella parvula (97,5%), Aggregatibacter actinomycetemcomitans (94,7%), Porphyromonas gingivalis (84,3%), Lactobacillus rhamnosus (84,3%).) e Actinomyces naeslundii (66,6%)
Guven et al., 2018 15	India	Clinical study	III	20 samples	Primary molars with acute infection	Fusobacterium nucleatum was the most frequent bacterium (100%), followed by Parvimonas micra (65%), Provetella intermedia (45%) and Treponema denticola (45%). Other species identified: Porphyromonas gingivalis, Tannerella forsythia, Campylobacter rectus.
Induja et al., 2019 16	India	Clinical study	III	20 samples	Endodontic infections	Of the 20 samples collected from endodontic infections, E. faecalis was isolated in 12 samples.
Zargar et al., 2020 17	Germany	Prospective cross-sectional study	SAW	41 samples	Root canal (irreversible pulpitis and primary endodontic infections)	Sixteen microbial species, 1 fungus (Candida albicans) and 1 virus (Herpes simplex virus) were discovered and isolated. Species with the highest prevalence: Dialister invisus (68.3%), Porphyromonas gingivalis (58.8%), Streptococcus salivarius (58.5%) and Treponema denticola (56.1%). Lysinibacillus fusiformis (19.1%) was detected in root canals for the first time. Candida albicans was observed in 11 cases (26.8%). Herpes simplex virus (HSV) was observed in 4 patients (9.8%).

Nardello et al., 2020 18	USA	Clinical study	III	5 samples	Root canals of teeth with apical periodontitis	A taxa bacteriana Bacteroidales [G-2] bactéria HMT 274, Porphyromonas endodontalis, Tannerella forsythia, Alloprevotella tannerae, Prevotella intermedia, Pseudoramibacter alactolyticus, Olsenella sp. HMT 809, Olsenella sp. HMT 939, Olsenella uli e Fusobacterium nucleatum subsp. animalis foram dominantes.
Gomes et al., 2020 19	United Kingdom	Clinical study	III	100 teeth	Root canals of teeth with primary and persistent/secondary endodontic infection	F. alocis was detected in 23 and 28 primary endodontic infections and 12 and 11 secondary endodontic infections, using Nested PCR and qPCR, respectively.
Puletic et al., 2020 20	Japan	Clinical study	III	39 periodontal abscesses; 33 necrotizing ulcerative periodontitis; 27 chronic periodontitis and 30 with healthy periodontal tissue	Periodontal disease is healthy and in different forms of periodontal disease.	Significantly higher detection rates of P. gingivalis, T. forsythia, and P. intermedia than in healthy individuals. The presence of cytomegalovirus was more frequent in patients with periodontal abscess.
Zargar et al., 2019 21	Iran	Clinical study	III	30 patients with secondary endodontic infection	Root canals of teeth with secondary endodontic infection	Tannerella forsythia foi a bactéria mais comum encontrada. Foram encontradas 13 espécies bacterianas (Treponema denticola, Streptococcus mitis, Porphyromonas gingivalis, Streptococcus salivarius, Prevotella intermedia, Tannerella forsythia, Enterococcus faecalis, Eikenella corrodens, Treponema parvum, Atopobium parvulum, Dialister invisus, Prevotella pallens, Fusobacterium nucleatum) uma cepa de vírus (HSV-1) e uma cepa de fungo (Candida albicans).
Chen et al.,	China	Clinical study	III	20 patients with periodont	Pus of the abscess, periodon	Porphyromonas gingivalis, Prevotella intermedia and other Prevotella spp. were the predominant bacteria of human

2019 22				al abscess and gingival folds from 25 healthy subjects	tal pocket coronally above the abscess and gingival sulcus of the healthy periodontal tooth.	periodontal abscesses. The abundances of Filifactor alocis and Atopobium rimae were significantly higher in periodontal abscesses than in periodontal pocket
Bottger et al., 2021 23	Germany	Clinical study	III	50 patients with severe odontogenic abscess	Saliva and pus	Polymicrobial infections were observed in 96% of cases; monoinfection in 4%. On average, 31.44 bacterial genera were detected in pus and 41.32 in saliva. A predominantly anaerobic bacterial spectrum was predominantly found in pus, while saliva showed an oral microbiome similar to healthy individuals.
Tzanetakis et al., 2022 24	Greece	Clinical study	III	60 teeth associated with pulp necrosis and apical periodontitis	Root canals	Aspergillus was detected in 8 cases, while C. albicans was detected in 4 cases. 35 samples showed evidence of bacterial growth. The vast majority were colonized by Achromobacter xylosoxidans followed by Lactobacillus spp., Methylobacterium spp. and Enterococcus faecalis.
Georgiou et al., 2023 25	Holland	Clinical study	III	29 teeth	Patients with primary or secondary apical periodontitis	The predominant genus in the entire sample set was Fusobacterium with relative abundance of 12.3%, followed by Prevotella (9.9%), Actinomyces (7.7%) and Streptococcus (6.7%).
Judith et al., 2022 26	India	In vitro cross-sectional study	SAW	50 samples	Pus from odontogenic abscess	Enterococcus faecalis (38.24%) followed by Staphylococcus aureus (29.41%) were identified, and the gram-negative bacilli that were isolated were Klebsiella pneumoniae (14.71%), Pseudomonas aeruginosa (8.82%), and Escherichia coli (5.88%).
Bernal-Trevino et al., 2018 27	Mexico	Clinical study	III	50 samples	Patients with primary and persistent endodontic	Of the 50 teeth evaluated, 18 of them (36%) had fungal infection. The predominant yeast species was Candida albicans.

					infection in 47 patients	

DISCUSSION

Infectious processes in the root canal system occur after necrosis of the pulp tissue, which may result from the progression of dental caries, trauma, periodontal disease, or even iatrogenic or failure of previously performed endodontic treatments. Thus, once the root canals are infected, the infection progresses to the periapex, and the pathogenic microorganisms and the infection spread28. The oral microbiota of patients with endodontic infectious processes, specifically odontogenic abscesses, is described as similar to that of healthy individuals; although, significant variations may exist, making the oral environment with greater pathogenic potential23. Different clinical studies in the literature show the polymicrobial nature of endodontic infections, and therefore, a solid understanding of the microbial composition of these infectious processes is essential for clinical decision-making, enabling a high-quality endodontic practice, based on scientific evidence28.

Primary endodontic infection, in general, is characterized by the presence of a wide microbial diversity, especially composed of anaerobic microorganisms29. As for the microbial composition, infectious processes of endodontic origin are mostly composed of anaerobic bacteria. Considering the states analyzed, the bacterium E. faecalis was one of the most frequently reported microorganisms. The bacterium Enterococcus faecalis is a facultative, Gram-positive anaerobic bacterium associated with antibiotic resistance, contributing to the risk of colonization and infection30. In addition, the presence of oral biofilm favors the colonization of these microorganisms, since antibodies cannot penetrate this biofilm[5].

In an analytical cross-sectional study conducted by Zargar et al., 201921, whose objective was to identify the microbial composition in persistent endodontic infections, based on culture and molecular biology methods using sequence analysis of the 16S rDNA gene, a higher prevalence of E. faecalis associated with root canals of teeth with apical periodontitis secondary to endodontic treatment was observed. These findings corroborate the other studies included in the sample, which sought to evaluate the microbiota existing in secondary endodontic infectious processes. The current literature indicates that Enterococcus faecalis is one of the most prevalent species in the root canal system of teeth with secondary/persistent apical periodontitis, corroborating findings [19,28,31-37].

In addition to E. faecalis, there is a wide range of anaerobic bacteria associated with endodontic infections. Genera of anaerobic gram-negative bacteria such as

Porphyromonas, especially the species Porphyromonas gingivalis; Dialister, Treponema, Fusobacterium, Prevotella, and Streptococcus were frequent findings in the studies included in our sample8,9,13,15,17,18,20,21,22,25,26. These findings are consistent with the evidence in the literature, which reports the dominance of bacteria of the genus Porphyromonas and Prevotella, especially in odontogenic abscesses22. In a literature review conducted by Siqueira and Roças, 202228, with the objective of analyzing important microbiological aspects of apical periodontitis and future perspectives, the authors corroborate our findings, reporting a high frequency of Streptococcus, Actinomyces, C. acnes, P. alactolytic, Arachnia propionica, Dialister, F. nucleatum, P. micra and genus Prevotella, in patients with post-treatment infections.

A factor of great impact that culminates in the expressive dominance of strictly or facultatively anaerobic bacteria in endodontic infections is due to the low availability or total deprivation of oxygen. The first microorganisms that appear in an endodontic lesion are anaerobic or facultative anaerobic, because the endodontic environment where they are inserted either has a low level of oxygen, or is totally deprived of this gas that is vital for aerobic beings. In addition, the presence of substrates that enable the nutrition of these microorganisms facilitates their colonization5.

In addition to bacteria, microorganisms from other kingdoms have been associated with infections of endodontic origin. Three (3) studies included in the sample identified species of the genus Candida, and one study identified the presence of Aspergillus in 8 cases followed17,21,27. Species of the genus Candida are part of the oral microbiota, having a commensal relationship with the organism. Evidence in the literature indicates that, although these microorganisms are aerobic, they can behave as facultative anaerobes38. In a recent systematic review conducted by Mergoni et al., 201839, with the objective of systematically reviewing the literature on the prevalence of Candida species in root canal infections, the authors concluded that, although the presence of Candida species associated with endodontic infections is uncommon, these microorganisms may have a relevant pathogenic role that may require adaptation of endodontic therapies with specific actions to control fungi.

Regarding viral composition, in the present study, 3 studies were identified that reported the presence of species of the order Herpesvirales, with cytomegalovirus and herpes simplex virus (HSV) being the ones reported17,20,21. Ozbek et al., 201640, through a clinical study, sought to investigate and compare the radiographic size of acute apical abscess lesions for the presence of Epstein-Barr virus (EBV), human cytomegalovirus (HCMV), human herpesvirus-6 (HHV-6), and human papillomavirus (HPV) DNA by means of the real-time polymerase chain reaction (PCR) method. The authors observed that HCMV was the most frequent herpesvirus in large and small apical abscess lesions; and that in large lesions, EBV and HHV-6 tend to occur in co-infection with HCMV. Similarly, in a clinical study conducted by Li et al., 200941, the authors concluded that EBV is very frequent in cases of irreversible pulpitis and apical periodontitis and that EBV, but not HCMV, HSV-1, or VZV, is associated with irreversible pulpitis and apical periodontitis and may potentially play a role in endodontic disease. Despite the studies, the evidence in the literature is still limited and heterogeneous, and therefore, more clinical studies with greater scientific evidence, with larger samples, are needed.

CONCLUSION

The present study found a dominance of Gram-negative and anaerobic bacteria in the microbial composition of infectious processes of endodontic origin. However, bacteria such as E. faecalis, Gram-positive, facultative anaerobic, can pose great risks, especially due to antibiotic resistance. In addition, evidence was observed in the literature regarding the composition of viruses and fungi in these infections; However, evidence is still scarce and, therefore, more studies of greater methodological rigor and sample size are needed.

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